Appln. No. 09/780,757
'Amd. dated November 15, 2004
Reply to Office Action of June 15, 2004

Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application:
Listing of Claims:

Claims 1-18 (Cancelled)

19. (Previously Presented) A method for determining binding of a species at a lipid-based surface having a local environment at a given pH or surface potential, wherein said binding is effective to alter said pH or potential, the method comprising:

incorporating at said lipid-based surface a probe which comprises a pH- or potential-sensitive fluorophore attached to a steroid, to a head group of a sphingolipid or to a head group of a lipid having at least two chains, each chain comprising at least 14 carbon atoms in length, and wherein each independently said chain is selected from the group consisting of acyl, alkyl or alkenyl, wherein incorporation of the probe at the lipid-based surface is substantially not altered upon binding or dissociation of the species at the lipid-based surface and

observing a change in a fluorescent property of said fluorophore retained at the surface upon binding or dissociation of said species at said lipid-based surface.

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- 20. (Previously Presented) The method of claim 19, wherein said lipid-based surface is the surface of a lipid bilayer.
- 21. (Previously Presented) The method of claim 19, wherein said fluorophore is selected from the group consisting of a pH-sensitive lissamine rhodamine compound, 7-hydroxycoumarin, fluorescein, and pH- or potential-sensitive derivatives thereof.
- 22. (Previously Presented) The method of claim 19, wherein said lipid is a phospholipid.
- 23. (Previously Presented) The method of claim 21, wherein said phospholipid is a diacyl, dialkyl or dialkenyl phosphatidyl ethanolamine or ceramide phosphoethanolamine.
- 24. (Previously Presented) The method of claim 23, consisting of 7-hydroxycoumarin conjugated via a 3-carboxamide linkage to the head group nitrogen of a diacyl, dialkyl, or dialkenyl phosphatidyl ethanolamine, or ceramide phosphoethanolamine.
- 25. (Previously Presented) The method of claim 19, wherein said species is a biomolecule having groups which are positively or negatively charged at a selected pH between about 2.0 and 12.0.

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- 26. (Previously Presented) The method of claim 25, wherein said groups are positively or negatively charged at a selected pH between about 4.5 and 7.5.
- 27. (Previously Presented) The method of claim 25, wherein said biomolecule is a nucleic acid.
- 28. (Previously Presented) The method of claim 25, wherein said biomolecule is a protein comprising amino acids with acidic or basic side groups.
- 29. (Previously Presented) The method of claim 19, wherein said surface comprises groups which are positively or negatively charged at a selected pH between about 2.0 and 12.0.
- 30. (Previously Presented) The method of claim 29, wherein said groups are positively or negatively charged at a selected pH between about 4.5 and 7.5.
- 31. (Previously Presented) The method of claim 20, wherein said lipid bilayer comprises a lipid having a cationic head group.
- 32. (Previously Presented) The method of claim 19, wherein, upon said incorporating, said fluorophore is separated from said surface by a distance equal to or less than 15 nm.

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- 33. (Previously Presented) The method of claim 19, wherein said lipid or steroid is attached to two or more fluorophores.
 - 34. (Cancelled)
- 35. (Currently Amended) The method of claim $34\underline{40}$, wherein said fluorophore is covalently bound to said polymer.
- 36. (Previously Presented) A method for determining binding of a species at a surface having a local environment at a given pH or surface potential, wherein said binding is effective to alter said pH or potential, the method comprising:

stably incorporating at said surface a probe which comprises a pH- or potential-sensitive fluorophore attached to a steroid, to a head group of a sphingolipid or to a head group of a lipid having at least two hydrophobic chains, each said chain comprising at least 14 carbon atoms in length, and

observing a change in a fluorescent property of said fluorophore upon binding or dissociation of said species at said surface.

37. (Currently Amended) The method according to claim 34-40 wherein the polymer is a cationic polysaccharide.

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- 38. (Previously Presented) The method according to claim 37 wherein the cationic polysaccharide is dextran.
- 39. (Currently Amended) The method according to claim 3440 wherein the fluorophore is selected from the group consisting of lissamine rhodamine, 7-hydroxycoumarin, fluorescein, and pH- or potential-sensitive derivatives thereof.
- 40. (New) A method for detecting binding of a test species to a given surface having a defined pH or surface potential, comprising:
 - a. stably incorporating a pH- or potentialsensitive fluorophore into or at the surface
 such that a change in pH or surface potential
 of the surface will result in a change in an
 observable property of the fluorophore;
 - b. bringing the test species into contact with the fluorophore-incorporated surface; and
 - c. observing any change in the observable property of the fluorophore, whereby a change of the observable property of the fluorophore indicates binding of the test species to the surface.

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- 41. (New) The method according to claim 40 wherein the surface is not a lipid-based surface.
- 42. (New) The method according to claim 40 wherein the surface is a polymer.